ON THE PROBLEMS OF INTESTINAL BACTERIOLOGY OF FOREST-LITTER CONSUMING INVERTEBRATES

I.M.Szabó, Chu Thi Loc, E.Contreras, M.Heydrich, K.Jáger, K.Marialigeti, M.Pobozsny, K.Ravasz, A.Zicsi
Departament of Microbiology and Systematic Zoology and Ecology, Eötvös L. University, Budapest

I.Szabó
Institute of Biology, University Medical School, Debrecen; Hungary

О ПРОБЛЕМАХ КИШЕЧНОЙ БАКТЕРИОЛОГИИ У ПОТРЕБЛЯЮЩИХ ЛЕСНУЮ ПОДСТИЛКУ ПОЧВЕННЫХ БЕСПОЗВОНОЧНЫХ

И.М.Сабо, Чу Тхи Лок, Э.Контрерас, М.Хейдрих, К.Ягер, К.Мариалигети, К.Побожны, К.Равас, А.Зичи
Кафедра микробиологии и кафедра зоосистематики и экологии Университета, Будапешт

И.Сабо
Институт биологии при Университете медицинской школы, Дебрецен; Венгрия

At present it is already a well documented fact that the interactions between soil invertebrates and their internal as well as external microbial partners play a decisive role in regulating the nutrient cycling processes in forest ecosystems /1,2,6/. Although there is a very extensive literature on the contribution of these interactions at gross level to the annual turnover of elements in many ecological systems studied, relatively little is known about the composition, nature and dynamics of gut floras of litter and soil feeding invertebrates. But considering the complexity of microenvironmental factors which govern the organization and activities of gut microbial communities, and the inadequacy of many of our methods used for studying them, this is not surprising al all. Below we present only some selected problems, which however arise frequently on studying the gut floras and the invertebrate-microbial interactions.

Serious Difficulties Arising on Taxonomic Identification of True Indigenous Gut Bacteria

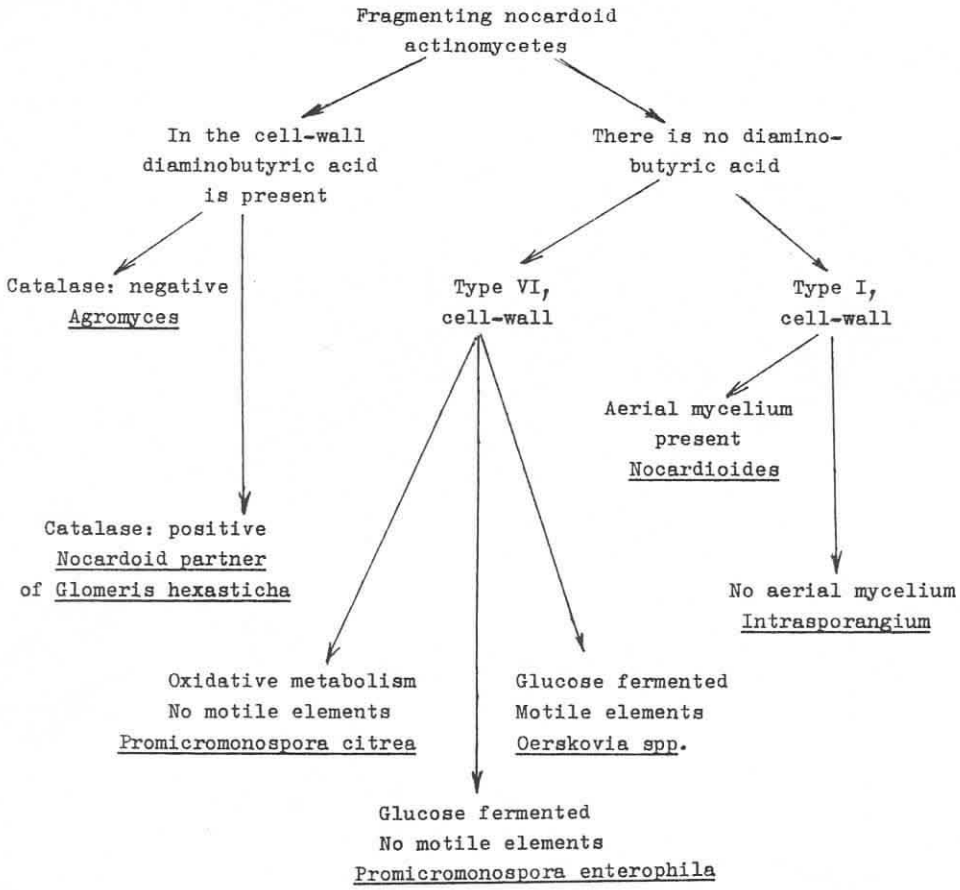
It is evident that any comparison between different intestinal microfloras, either on functional or structural bases, can be exactly carried out only with taxonomically identified isolates. But is this aim always attainable? Unfortunately not. The true indigenous intestinal bacteria of soil invertebrates are only partly identical with those of the vertebrates, but frequently they are less or even completely unknown organisms.

In the early eighties we showed that yellow coloured bacteroid smearable nocardioform actinomycetes producing fine Gram-positive hyphae fragmenting into rod shaped and coccoid elements and characterized by type VI. cell wall chemical composition are typical members of millipede gut floras /4,9/. The

first species, the strains of which were isolated from the gut and faeces of Chromatoiulus projectus (Diplopoda), was described by the collaborators of our Department as Promicromonospora enterophila /5/. Some workers, however, are of the opinion that this organism, owing to its ability to grow anaerobically and ferment glucose as well as to its other distinguishing diagnostic properties, does not belong to the otherwise less known Promicromonospora, but to a new genus. According to other workers, as regards the particular phage-sensibility spectra of our P.enterophila strains, it would be a new atypical Oerskovia species. But among others, also the existence of Oerskovia as a separate genus is still questionable.

In 1982, discussing the problems of millipede bacteriology at the VIII. International Colloquium on Soil Zoology in Louvain-la-Neuve (Belgium) Professor J.M.Anderson (Exeter) mentioned that on studying the microflora in the hindgut of Glomeris specimens by electronmicroscopic observations, he detected the presence of monosporic actinomycetes. In the following year, in our Department, Chu Thi Loc isolated from the gut and faeces of Glomeris hexasticha an unique nocardioform monospore actinomycete. All of the studied strains of this gut actinomycete proved to be morphologically and biochemically identical with each another. Accordingly, in the gut of this millipede exists a homogeneous population of only one nocardioform species which can be clearly distinguished from the nocardioform partner (P.enterophila) of Ch.projectus. This organism shows a very complex life cycle involving oval or sphaerical spore-like bodies, chlamydospores, rod-shaped solitary vegetative cells or hyphal bits, unseptated extensively branching substrate hyphae, swollen vesicles and subterminal vesicular bodies. Our G.hexasticha nocardoid-strains proved to be catalase, urease and phosphatase positive, cellulase and oxidase negative. They hydrolysed Tween 80, tributyrin, aesculin, tyrosine and gelatin. They did not survive heat treatment at 70°C for 4 hr, but grew well at pH 11.

They were sensitive to penicillin (310), oxacillin (10 µg) and vancomycin (50 µg). At the first taxonomic approach to this nocardioform gut population of G.hexasticha, we supposed that it represented an intermediary taxon between the genera Oerskovia and Promicromonospora. This supposition seemed to us the more acceptable because the life cycle of our strains resembled greatly that of P.citrea /7/. This idea was, however, not corroborated by the result of our cell wall analyses, which revealed that this gut actinomycete is characterized by a particular cell-wall chemical composition (diaminobutyric acid and glycine are present) and its whole cell sugar pattern is type C. In the final analysis, the actinomycete partner of G.hexasticha proved to be unidentifiable also at generic level because the combination of diaminobutyric acid, oxidative metabolism, substrate mycelium only, production of vesicles, and monospore-like bodies is lacking among the actinomycete genera recognized so far (Fig.). To introduce it as a new genus would not offer a problem-free solution either. Nevertheless, at present we have very few data about the range of variability of nocardoid genera, and the taxonomic significance of the cell-wall chemical composition is also questioned by some workers. All these clearly show that the correct identification of an indigenous gut microbe frequently encounters great difficulties.



Tentative systematic position of nocardoid actinomycete strains isolated from faecal matter of G.hexasticha among the closely related nocardoid genera

The Question of the Presence and Activities of True Intestinal Bacteria in the Soil Environment of the Host Animal's Feeding Habitat

The population densities of soil invertebrate gut- or faecal-floras and those of the soil or litter matters themselves of the host animals' feeding habitats were in many cases subjected to comparative studies. The published results clearly showed that the total number of bacteria is in the hind gut or the faeces of millipedes, woodlices, fly larvae, earthworms, etc. many times larger than in the soil, litter, etc. consumed as food matter by them. On the qualitative differences in the composition of these bacterial floras, however, we have only very few information.

The established gut bacterial biota of soil invertebrates is generally, composed at any time, of two large fractions /3/: a, certain common soil bacteria, which can multiply also in the animal's digestive tract. b, true intestinal bacteria among which there are more or less known enterobacteria of vertebrates, and particular less known or unknown gut bacteria characteristic of individual species or groups of invertebrates.

Using the International Streptomyces Project (ISP)-criteria and -methods, in this year, we detected the codominant occurrence of such typical soil streptomycetes in the gut of earthworms (Octolasium montanum, Fitzingeria platyura depressa, Allolobophora rosea, adult and juvenile specimens of Lumbricus polyphemus) as are Streptomyces olivaceus, S.antibioticus, S.longisporoflavus, S.violaceoruber, etc. A computer analysis performed also in this year by us on the complex intestinal flora of the millipede Cylindroiulus boleti revealed that the soil bacteria Micrococcus roseus and Bacillus cereus can attain prominent community position among the indigenous intestinal colonizers of this animal. Of course, many other soil bacteria do not survive the intestinal passage, or at least pass inactively through the digestive canal.

At present most data are also in the literature, at our disposal about the occurrence of true ubiquitous enterobacteria in the gut of soil invertebrates. E.g. in the last years we isolated from the fresh faeces of the millipede Chromatoiulus projectus a lot of Klebsiella sp., Enterobacter sp. and Pseudomonas variants. Earlier we obtained Enterobacter aerogenes strains from the hind gut of Bibio marci larvae. About the survival in soils and the patterns of transmission to the young animals of these enterobacteria we have, however, very few data or none at all. The data obtained on the particular gut bacteria of individual soil invertebrate species or groups are more sporadic. During the last two years, we carried out many series of laboratory experiments and analysed natural soil microbiotas to clarify the factors which influence the survival of Promicromonospora enterophila in the forest environment. Our summarized results are presented in the Table.

As can be seen, it is impossible to survive for this true gut actinomycete in the soil (A_F-horizon matter) of the feeding habitat of its host animal, in the presence of the complex natural microflora at a water content of about pF 2, which is in general the most advantageous for intense microbial activities. Under such conditions P.enterophila completely disappears from the soil within only 1-2 months, and this process of elimination is stimulated if the activities of the microflora is increased by adding glucose, peptone, yeast extract, etc. to the soil. This is the reason why also in natural soils which are densely colonized by the host millipede, P.enterophila occurs only sporadically or not at all. E.g. among 1582 random isolates obtained from a composite sample of the A_F-horizon (feeding habitat of Diplopoda) of a brown forest soil (W.Hungary), Promicromonospora-Oerskovia type microbe was not detectable at all. In other cases, from among such a large number of isolates only one or two proved to be Promicromonospora.

In all cases when we decreased the antagonistic potential of the complex natural soil microflora, e.g. at very low water content (pF 6.0), the survival ability of this organism increased considerably, and in sterile soil

Survival of Promicromonospora enterophila-strains in A_F-horizon matter of an eutrophic brown forest soil under experimentally modified environmental conditions*

	Experimental conditions	Duration of survival
1	Natural soil conditions at a water content of about pF 2	1-2 months
2	At a soil moisture of about pF 6	> 8 months
3	In soil enriched with 1% glucose at pF 2	1-2 months or less
4	In soil enriched with 1% yeast-extract at pF 2	very short (only weeks)
5	In heat sterilized soil at pF 2	>1 year
6	In heat sterilized, air dried soil	>1 year
7	In heat sterilized soil (pF 2), together with Aspergillus niger	>8 months
8	In heat sterilized soil (pF 2), together with a Penicillium sp.	>8 months

* The ability to survive under the given environmental conditions was checked by extensive reisolations after exposure.

it could survive for more than one year without changing its biochemical activity.

Concentrating research more intensively also on the qualitative taxonomic and functional differences between the invertebrate gut and soil bacterial floras is likely to lead to more rapid advances in this field of soil zoology.

R e f e r e n c e s

1. Anderson J.M., Macfadyen A. (eds). The Role of Terrestrial and Aquatic Organisms in Decomposition Processes. Oxford: Blackwell Scientific Publications, 1976.
2. Ausmus B.S., Edwards N.T., Witkamp M. Microbial immobilisation of carbon, nitrogen, phosphorus and potassium: implications for forest ecosystem processes // The Role of Terrestrial and Aquatic Organisms in Decomposition Processes / Ed. J.M. Anderson, A.Macfadyen. Oxford: Blackwell Scientific Publication, 1976. P. 397-419
3. Baleaux B., Vivares Ch.P. Étude préliminaire de la flore bacterienne intestinale de Schizophyllum sabulosum var. rubripes Lat. (Myriapoda, Diplopoda) // Bull. Soc. Zool. France. 1974. Vol. 99. P. 771-779.
4. Dzingov A., Márialigeti K., Jáger K., Contreras E., Kondics L., Szabó I.M. Studies on the microflora of millipedes (Diplopoda). I. A comparison of actinomycetes isolated from surface structures of the exoskeleton and the digestive tract // Pedobiologia. 1982. Bd. 24. S. 1-7.
5. Jáger K., Márialigeti K., Hauck M., Barabás G. Promicromonospora enterophila sp. nov., a new species of monospore actinomycetes // Int. J. Syst. Bact. 1983. Vol. 33. P. 525-531.

6. Krivolutzsky D.A., Pokarzhevsky A.D. The role of soil animals in nutrient cycling in forest and steppe // Soil Organisms as Components of Ecosystems / Eds. U.Lohn, T.Persson. Ecological Bulletin 25. Stockholm: Swedish Natural Science Research Council, 1977. P. 253-260.
7. Kuimova T.F., Yevtushenko L.I., Agre N.S. The characteristics of Promicromonospora citrea life cycles // Microbiology. 1983. Vol. 52. P.268-276.
8. Lechevalier H.A., Lechavalier M.P. Introduction to the order Actinomycetales // The Prokaryotes. A Handbook of Habitats, Isolation and Identification of Bacteria / Eds. M.P.Starr, H.Stolp, H.G.Trüper, A.Balows, H.G.Schlegel. B., Heidelberg; N.Y.: Springer-Verlag, 1981. P. 1915-1922.
9. Szabó I.M., Jáger K., Contreras E., Márialigeti K., Dzingov A., Barabás G., Pobozsny M. Composition and properties of the external and internal microflora of millipedes-Diplopoda // New Trends in Soil Biology /Eds Ph.Lebrun, H.M.André, A. de Medts, C.Gregoire-Wibo, G.Wauthy. Louvain-la-Neuve: Dieu-Brichart. 1983. P. 197-206.